SYNOPSIS FOR REGISTRATION OF Ph.D

On

Assessment of Hypolipidemic and antiobesity potential of extracts and fractions of plants and formulations using animal model

Submitted

To

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Abstract

Hyperlipidemia is caused by lifestyle habits and food practices. Lifestyle contributed includes obesity, not exercising, and smoking. Conditions that cause hyperlipidemia include diabetes, kidney disease, pregnancy, and an under active thyroid gland. Among the various fetal diseases cardiovascular disease is also one of the major disorders which have higher mortality rate over the world. Hyperlipidaemia is associated with risk factors like hypertension, Type II Diabetes mellitus, obesity, myocardial infarction, congestive cardiac failure, angina pectoris, gallbladder diseases, degenerative joint diseases, sleep apnea, and infertility. Synthetic drugs are available for counteracting liver injury and hyperlipidaemia, but the side effects and cost associated with these modern drugs necessitates the search for alternative which are without or minimize side effects and cost effective. Number of plants have traditional claims for antihyperlipidemic potential but still not entirely explored. The present investigation will assess a few plants for hypolipidemic and antiobesity potential by using appropriate screening models. Suitable polyherbal formulation will also be screened for dyslipidaemia and obesity.

1. Introduction:

In proper quantities, lipids, which include cholesterol and triglycerides are an important part of a body's maintenance, chemistry and overall health. Having higher levels of lipids, i.e. hyperlipidemia, may lead or contribute to blocked arteries and an increased chance of heart disease or stroke. Cholesterol is an organic molecule found in our blood that helps to break down nutrients and create hormones. LDL is considered the "bad" form of cholesterol, as it's the form that builds up within your arteries and can lead to cardiac complications. Hyperlipidemia is a common predicament in society due to changes in lifestyle and food practice[1]. Hyperlipidemia is a highly predictive risk factor for atherosclerotic coronary artery disease and cerebral vascular diseases[2]. Primary hyperlipidemia, which is usually, takes place as a result of genetic problems, i.e., a mutation within receptor protein, while secondary hyperlipidemia will arise as a result of other underlining diseases like diabetes.

CVD was the largest cause of deaths in males (20.3%) as well as females (16.9%) and led to about 2 million deaths annually. 73.5 million adults (31.7%) in the United States have high, low-density lipoprotein (LDL), or "bad," cholesterol[3]. The Global Status on Non-Communicable Diseases Report 2011 has reported that there were more than 2.5 million deaths from CVD in India in 2008, two-thirds due to CHD and one-third to stroke[4]. The mortality is highest in south Indian states, eastern and north-eastern states and Punjab in both men and women, while mortality is the lowest in the central Indian states of Rajasthan, Uttar Pradesh and Bihar[5]. In 2002, CVDs contributed to approximately a third of entire global deaths, whereas, by the year 2020, it is expected that CVDs will become the leading cause of death and disability worldwide.

Today in most of the developed and developing countries, hyperlipidemia and thereby atherosclerosis are the leading cause of cardiac illness and deaths. Several synthetic hypocholesteromic agents such as statins, fibrates, resins and nicotinic acid are capable of efficiently reducing plasma total cholesterol (TC) levels, lower blood cholesterol level, either by inhibiting endogenous synthesis and/or by lowering cholesterol absorption from the intestine but LDL does not undergo any significant alteration and the search for new drugs capable of reducing and regulating serum cholesterol level has gained interest resulting in numerous reports on significant activities of natural agents. Also, synthetic hypolipidemic agents have one or more side effects and are unable to increase HDL levels. Recent studies have shown that many compounds of herbal origin are able to reduce plasma TG and TC levels and elevate HDL[6, 7]. These attributes in reducing the risk of CHD The major portion of the global population in developing countries still relies on botanical drugs to meet its health needs.

The attention paid by health authorities to the use of herbal medicines has increased considerably, both because they are often then only medicine available in less developed areas and because they are becoming a popular alternative treatment in more developed areas. In the recent year, the interest in medicinal plants has been increasing due to the higher side effects and adverse effects of allopathic drugs. The major side effects of antihyperlipidemic agents include muscle toxicity, rhabdomyolysis, psychiatric adverse reactions which include depression, memory loss, hepatic dysfunction, renal insufficiency, hypothyroidism, advanced age and serious infections, confusion and aggressive reactions[8, 9]. The most prominent side-effect of fibrate is myositis, which commonly occur when renal function is impaired or statins are given. The concept of herbal medicine focuses on balancing the humor of the body and they are used to treat various ailments.

Medicinal plants are the “backbone” of traditional medicine, which means more than 3.3 billion people in the less developed countries utilize medicinal plants on a regular basis. Thus, there is a considerable interest in the development of lipid lowering drugs from natural products in the recent years. Plant stanols or sterols, flavonoids and other polyphenols have been shown its beneficial effect in controlling hyperlipidemia. Thus, herbal medicines have been given a valuable status by the WHO and have endorsed their safe and effective use[10, 11]. Therefore, the present study will evaluate various plants for their hypolipidemic and antiobesity potential by using various animal screening models. It will be used to treat various complications due to hyperlipidemia and reduce mortality rate owing to hyperlipidemia by using natural constituents with the lowest amount of side effects and cost effectiveness.

2. Selection of herbal Plants:

There are number of plants screened for its hypolipidemic potential but the not fully explored. Therefore, the study will be conducted on those plants which are not fully studied for its hypolipidemic and antiobesity potential of plants extract and various fractions as well as various suitable polyherbal formulations of these plants parts. The plant will be selected on

the foundation to treat various signs and symptoms as well as cardio-complexity, dyslipidaemia and other complications related to hyperlipidemia. These plants having traditional claims for treating dyslipidaemia obesity but not explore. The plants are as *Sesamum species*. (Ranteel), *Tinospora cordifolia* (Gulvel), *Psoralea corylifolia* (Babchi, Bavachi, Bavanchi), *Trigonella foenum graecum* (Methi, Fenugreek), *Trachyspermum ammi* (Ajwain), *Bunium bulbocastanum* (Kala jeera, Shahi jeera), Dorali leaves. By using variety of plants suitable polyherbal will be formulated and screen for dyslipidaemia, obesity and other complications.

3. Review of literature:

The literature shows the hypocholesterolemic effect of *Allium cepa* L., *Allium sativum* L., *Glycine max* (L.), *Trigonella foenum-graecum* L., *Moringa oleifera* Lam., *Sesamum indicum* L., *Luffa species* and variety of plants from *Cucurbitaceae* family have traditional claims and reported potent antihyperlipidemic potential. Onion contains many sulfur containing active principles, which takes part in biological activities, mainly in the form of cysteine derivatives, namely, S-alkyl cysteine sulfoxides that decompose into a variety of thiosulfinates and polysulfides by the action of an enzyme allinase on extraction[12]. The essential oil fraction of garlic comprises 60% of diallyl disulphide (DADS) which showed hypocholesterolemic activity[13]. Fibre of soy bean showed maximum plasma cholesterol lowering effect in hypercholesterolemic male albino rats compared with fibres of cereals and pulses isolated from the seed coat when administered for 4 weeks[14]. The hypocholesterolemic effect of sesame seed powder administered (5% and 10%) to hypercholesterolemic male albino rats showed decline in plasma LDL-C level, increase in hepatic HMGCoA reductase activity and enhanced faecal bile acids and cholesterol excretion. The hypocholesterolemic effect appeared to be due to its fibre, sterol, polyphenol and flavonoid contents[15].

Hypolipidemic effect of fenugreek seeds and its comparison with atorvastatin on experimentally induced hyperlipidemia. The results showed that atorvastatin showed more potent hypolipidemic activity. It reduces serum total cholesterol, TG, LDL and VLDL, cholesterol, and the atherogenic index (LDL-C/HDL-C; $p < 0.001$) highly significantly as compared to fenugreek. And concluded that Fenugreek and atorvastatin both have hypolipidemic activity in rabbits but atorvastatin is more potent than fenugreek seeds powder[16]. Hypolipidemic effect of fenugreek seeds is mediated through inhibition of fat accumulation and upregulation of LDL receptor. Results illustrate in decrease of serum TG, LDL-cholesterol (LDLc), and body weight in a dose- and time-dependent manner ($P < 0.05$). Results indicate that hypolipidemic effect of TEFS is due to inhibition of fat accumulation and upregulation of LDLR[17].

The effect of fenugreek Seeds Powder (*Trigonella foenum-graecum* L.) on experimental induced hyperlipidemia in rabbits and results discovered that the aqueous emulsified fenugreek (TFG) seeds powder reduced significantly the serum total cholesterol, low-density lipoprotein cholesterol (LDL-C), and the atheriogenic index ($P < 0.001$), with

significant increase in the HDL-C ($P < 0.001$) in experimentally hyperlipidemic rabbits ($P < 0.001$) as compared to control group, whereas no significant differences were observed in the triglyceride very lowdensity lipoprotein cholesterol (VLDL-C) and body weight ($P > 0.05$) of both groups[18]. Efficiency of black cumin seeds on hematological factors in normal and hypercholesterolemic rabbits and result revealed that it significantly decreased WBC counts in the hypercholesterolemic group[19].

Antiobesity, antihyperlipidemic and hypoglycemic effects of the aqueous extract of *Nigella Sativa* seeds (Kalongi). Blood glucose, serum cholesterol, triglycerides and low density lipoproteins (LDL) were significantly decreased and high density lipoprotein HDL was significantly increased. On the basis of these results and the previous studies we can suggest the use of black seed for its role in weight reduction and holistic effects on health[20]. Anti-hyperlipidemic activity of fenugreek (*Trigonella foenumgraecum*) seeds extract in triton and high fat diet induced hyperlipidemic model: a potent anti-atherosclerotic agent. The aqueous seed extract at the same dose level significantly attenuated the elevated plasma total cholesterol, triglycerides, lipoprotein cholesterol (HDL, LDL and VLDL) in high-fat diet-induced hyperlipidemic rats. It also found to improve the atherogenic index (AI) in high fat diet model[21].

The comparative antihyperlipidaemic efficacy of *Trachyspermum ammi* extracts in albino rabbits and Simvastatin was used as a synthetic cholesterol lowering drug. The results suggested that chloroform and water extracts of *T. ammi* seed had no hypolipidaemic activity. However, methanol and petroleum ether extracts equivalent to its 2 g/kg body weight powder and Simvastatin (0.6 mg/kg body weight) were equally effective in treating hyperlipidaemia in albino rabbits[22]. Hypoglycemic effect of celery seeds (Ajwain / Ajmod) in streptozotocin induced diabetic rats and the results show Celery seeds extract treatment caused a statistically significant decrease in the elevated serum glucose levels and increase in the serum insulin concentrations in test group C as compared to group B[23].

Sesame (*Sesamum indicum* L.) protein isolate in rats fed a normal and high cholesterol diet and results indicate that sesame protein isolate decreases cholesterol concentration in plasma, increases HDL-cholesterol, and also decreases plasma and erythrocyte membrane lipid peroxidation with or without cholesterol fed diet in rats[24]. The impact of N-acetylcysteine and sesame oil on lipid metabolism and hypothalamic-pituitary-adrenal axis homeostasis in middle-aged hypercholesterolemic mice and showed prohibited the onset of the disturbed lipid profile, exhibiting decreased lipid peroxidation and alkaline phosphatase (ALP) levels, restored nitric oxide bioavailability and reduced hepatic damage, compared to non-supplemented groups[25]. Ajwain with its characteristic aromatic smell and pungent taste is widely used as a spice in curries. Its seeds are used in small quantities for flavoring numerous foods, as preservatives, in medicine and for the manufacture of essential oil in perfumery. In Indian system of medicine, ajwain is administered for curing various ailments. *T. ammi* has been shown to possess antimicrobial, hypolipidemic, digestive stimulant, antihypertensive effects[26].

4. Objectives of research:

- To prepare various plant extracts and fractions for checking antihyperlipidemic potential and evaluated for preliminary phytochemical constituents.
- To screen antihyperlipidemic potential of various herbal plant extracts.
- To screen the potent plant fractions of various herbal plants for their antihyperlipidemic activity.
- To screen activities of sub-fractions of the potent fractions
- To evaluate hypolipidemic potential of isolated molecules from potent fractions
- To prepare various polyherbal formulations.
- To screen the polyherbal formulation for evaluation of antihyperlipidemic activity.
- To prepare a final polyherbal formulation for loosening of weight and evaluate by using various methodologies.

5. Methodology:

a) Collection of different parts of the plants and authentication of plants.
b) Extraction of plant parts by using various solvents and extraction processes.
c) Phytochemical screening of different extracts and fractions using standard tests.
d) Screening of extracts by using in-vivo animal models for hypolipidemic potential.
e) Fractionation of potent extracts of plants for further evaluation of antihyperlipidemic potential.
f) By using the above evaluation data, preparation of different herbal formulations to treat hyperlipidemia, obesity and cardiocomplex disorders.
g) Screening of these herbal formulations by using various in-vivo animal models.

5.1 Experimental models

5.1.1 Triton-X induced hyperlipidemia: (Acute model)

Principle: This model is widely used in order to screen natural or synthetic drugs. Triton WR-1339, a non-ionic detergent (Oxy ethylated tertiary Octyl phenol formaldehyde polymer) can cause acute hyperlipidemia. It blocks the clearance of triglyceride rich lipoproteins to induce acute hyperlipidemia in several animals[27, 28, 29]. Triton WR-1339 has a direct inhibitory effect on the lipoprotein lipase in muscle and adipose tissue. This property of Triton WR-1339 is the basis for its extensive usage as a model of acute hyperlipidemia.

On 8th day or after 24 h of induction, blood samples will collected from all animals by retro orbital puncture under slight anesthesia and serum will separated and used for biochemical estimations such as high density lipoprotein (HDL), triglycerides (TG), total cholesterol (TC) will be estimated by using commercial kits. Very low density lipoproteins (VLDL), low density lipoproteins (LDL), TC/HDL, LDL/HDL will be calculated using the Friedewald formula.

5.1.2 High fat diet (HFD) induced hyperlipidemia: (Chronic Model)

Principle: Hypercholesterolemia in rodents is induced by supplementing cholesterol or saturated fats into laboratory rodent[30]. Excessive cholesterol feeding leads to susceptibility to hypercholesterolemia and arteriosclerosis. High dietary fat intake will find to promote the development of obesity and hyperlipidemia in both humans and rodents by altering the plasma cholesterol and triglyceride levels[31, 32], which could then lead to a higher risk for various metabolic syndromes, including cardiovascular diseases, fatty liver disease, dyslipidemia and type 2 diabetes mellitus[33, 34].

After the treatment blood samples will be collected from animals by different methods and serum will be separated and used for histological and biochemical estimation of HDL, TG, and TC will be estimated by using commercial kits. Liver will isolated from animals and estimated for total lipids in the liver. Also various sections of coronary arteries and caratoid arteries will be evaluated for assessment of effect of plant extracts on of lipid deposition. Glucose tolerance test will be performed. SGOT, SGPT tests for liver enzymes will be performed. And suitable in-vitro models will also be screened for hyperlipidemia, dyslipidaemia and obesity.

6. Proposed works Plan:

First Year:

- Collection of plant material, identification of plants.
- Preparation of extracts by using various solvents, procurement, drying of extracts
- Phytochemical screening of extracts
- Toxicity evaluation, preliminary studies and main studies.

Second year:

- Fractionation of potent extract
- Antihyperlipidemic activity of selected fractions
- Separation and isolation of compound by TLC, HPTLC, column chromatography

Third year:

- Preparation of polyherbal formulation
- Evaluation of antihyperlipidemic potential of selected polyherbal formulation and comparison with standard marketed products.

7. Significance of study:

- The study will be supportive to treat the various complications due to hyperlipidemia and obesity in patients.
- Plant based medicine is expected to have fewer side effects as compared to the modern drugs.
- The present study will be helpful to minimize the cost of the treatment of diseases due to hyperlipidemia.
- This study uses the plants as medicines which have been not fully explored and that are why it will be very important to investigate the antihyperlipidemic potential of these plants.
- This study functional to isolate the potent component from plants which have maximum antihyperlipidemic effect as compared to the synthetic drugs.

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